



Cardiometabolic risk factors and mercury content in hair of women from a territory distant from mercury-rich geochemical zones (Cherepovets city, Northwest Russia)

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Abstract For the first time in the Russian Federation, a study was conducted to determine the level of mercury in the hair of young and middle-aged women with or without metabolic syndrome (MS) and the Association of mercury content in hair with cardiometabolic risk factors. The studied cohort consisted of 387 women (204 with metabolic syndrome and 183 without metabolic syndrome as controls). The mercury content in the hair was determined by atomic absorption pyrolysis. The relationship between

mercury in hair and cardiometabolic risk factors was evaluated using linear regression. Calculation of the odds ratio (OR) of the risk of metabolic syndrome among quartiles of mercury content in the hair was performed by multiple logistic regression. Hg concentrations in hair were higher in women with metabolic syndrome than without metabolic syndrome (Mean – 520; median – 360; versus Mean – 330; median – 260 µg/kg, $P < 0.001$). There is an increased risk of developing cardiometabolic syndrome in women, whose hair contain over 544 µg/kg of mercury. The results of this study indicate that the intake of Hg in the human body can be one of the factors that can increase the development of cardiometabolic risk.

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Introduction

Mercury is an element present in the air, water, soil and being accumulated in living organisms (WHO 2017). Mercury (Hg) enters the environment from both natural and anthropogenic sources (Liu et al. 2012). Hg exists in various forms: elemental, inorganic and organic. The most dangerous compound for

living organisms is mercury in its methylated form, which is formed during microbiological processes in the aquatic environment. It migrates through the trophic network, reaching maximum concentrations in fish present in the diet of warm-blooded animals, including humans (Miklavcic et al. 2013; Rice et al. 2014). People can be exposed to mercury in any of its forms. However, exposure is mainly through the consumption of fish and shellfish contaminated with methyl mercury (Liu et al. 2012) and through the inhalation of elemental mercury vapors by workers during industrial processes (WHO 2017).

The Vologda Oblast (North-West of Russia) are located in the northern part of the Russian Plate within the East European ancient platform (Geology of the USSR 1963). Geologically, the region belongs to the territory where there are no deposits and ore occurrences of mercury (weakly mineralized territory) (Babkin 1976). On the territory of the Vologda region is the city of Cherepovets, where there are large industrial enterprises that burn a large amount of natural hydrocarbons in the industrial processes, which are potential sources of mercury entering the environment. An increase in mercury emissions from Cherepovets into the regional ecosystem is evidenced by high levels of mercury in bottom sediments, fish and terrestrial mammals of the upper trophic levels (Udodenko et al. 2018; Haines et al. 1992; Komov et al. 2016).

The most dangerous mercury compound is its methylated form, which is formed during microbiological processes mainly in the aquatic environment. It migrates through the trophic network, reaching maximum concentrations in fish present in the diet of warm-blooded animals, including humans (Miklavcic et al. 2013; Rice et al. 2014). It has been established that the main sources of methylmercury in the human body are both fresh water and salt water fish (Liu et al. 2012). Thus, consuming fish from local water bodies, the population of the Cherepovets is at risk of mercury accumulation (In Vologda Oblast the average consumption of fish products per capital is 23.3 kg / year (Household food 2017).

The Agency for Toxic Substances and Disease Registry (ATSDR) classifies mercury among ten most dangerous substances (ATSDR 2019). Exposure to mercury, even in small doses, causes serious health problems, producing a toxic effect on the nervous, digestive, immune, cardiovascular systems and poses

a threat to fetus development and the development of the child in the early stages of life (NAS 2000; WHO 2003; Houston 2011; Liu et al. 2012; Rice et al. 2014; Bell 2017). In children exposed to mercury in the prenatal period, there is a decrease in intelligence, impaired attention, memory, speech and motor functions (Pichery et al. 2012; ATSDR 2019).

Earlier studies have shown that mercury causes functional disorders in the vascular endothelium, enhances oxidative stress, causes hyperplasia of the smooth muscles of blood vessels and accelerates the process of atherosclerosis of the carotid arteries; it has been shown that the risk of high blood pressure and cardiovascular disease increases along with an increase in the mercury content in hair (Yorifuji et al. 2010; Kishimoto et al. 1995; Houston 2011).

Mercury, in addition to genetic factors and lifestyle factors, is one of the factors that affects the development of metabolic syndrome (Lind et al. 2013). Metabolic syndrome (also known as the cardiometabolic syndrome) refers to a cluster of related risk factors for cardiovascular disease that includes abdominal obesity, diabetes, hypertension and elevated cholesterol. People with the cardiometabolic syndrome have twice the likelihood of developing and dying from cardiovascular disease compared to those with no cardiometabolic risk factors (Benetos 2008; Suzuki 2008; Wilson 2005). Metabolic syndrome represents the overall risk of cardiovascular disease due to a number of modifiable and unmodifiable factors and markers (Glezer et al. 2010; Kanodia 2020). Modifiable (controlled) factors include smoking, hypodynamia, dyslipidemia, hypertension, increased blood glucose, overweight, obesity, insulin resistance, hypercoagulation and markers of inflammation; unmodifiable (unrecoverable): age, gender, race, hereditary predisposition to the development of cardiovascular disease (Gaysin et al. 2009). Previous findings indicate that people with a high level of heavy metals, especially Hg, are more likely to develop metabolic syndrome (Park et al. 2009).

The purpose of this study was to identify the relationship between levels of mercury in hair and cardiometabolic risk factors in young and middle-aged women in the city of Cherepovets, Vologda Oblast. The level of mercury in the hair of women and its relationship with risk factors for the development of cardiovascular diseases and metabolic syndrome have not been previously conducted in Russia.

Materials and methods

Hair samples were taken in 2018–2019 in medical institutions of the city of Cherepovets (Vologda Oblast, North-West of the European part of Russia: 59° 07' N 37° 54' E) (Fig. 1).

The collection of material was carried out in medical institutions of the city of Cherepovets, where health workers established the presence or absence of metabolic syndrome. The study involved 387 women aged 18 to 59 years, of which 204 had signs of metabolic syndrome and 183 without metabolic syndrome as controls. Medical staff completed questionnaires which included data on age, gender, place of residence, the presence/absence of cardiovascular disease, body mass index (BMI), blood pressure and biochemical blood indices (glucose and cholesterol).

The program of this study was discussed and approved by the Bioethics Commission of Cherepovets State University and the Territorial Department of Health of the Vologda Oblast (No. 2–1 / 55, 18.01.2019). All participants gave free informed written consent in accordance with the world medical Association code of ethics (Helsinki Declaration) to conduct experiments with human participation

(Williams 2015) and to publish the results obtained. Only adults participated in the study. The participants' privacy rights were of paramount importance.

According to the recommendations of the World Health Organization—WHO (1990), International Federation of Diabetologists—IDF (2005), American experts of the National Cholesterol Education Program—NCEP ATP III (2003), the presence of metabolic syndrome was assessed by the following diagnostic criteria (Kalashnikova 2013; Poursafa et al. 2014):

1. Body mass index (BMI), $\text{kg/m}^2 > 30$;
2. Blood pressure, mmHg: diastolic blood pressure, DBP (minimum pressure between two heartbeats) ≥ 85 ; systolic blood pressure, SBP (maximum pressure during one heartbeat) ≥ 130 ;
3. Fasting glucose, mM/L ≥ 5.6 ;
4. Cholesterol, mM/L ≥ 5.2 .

Women who had at least three criteria for the diagnosis of metabolic syndrome belonged to the group with metabolic syndrome, all the rest to the group without metabolic syndrome (Poursafa et al. 2014).

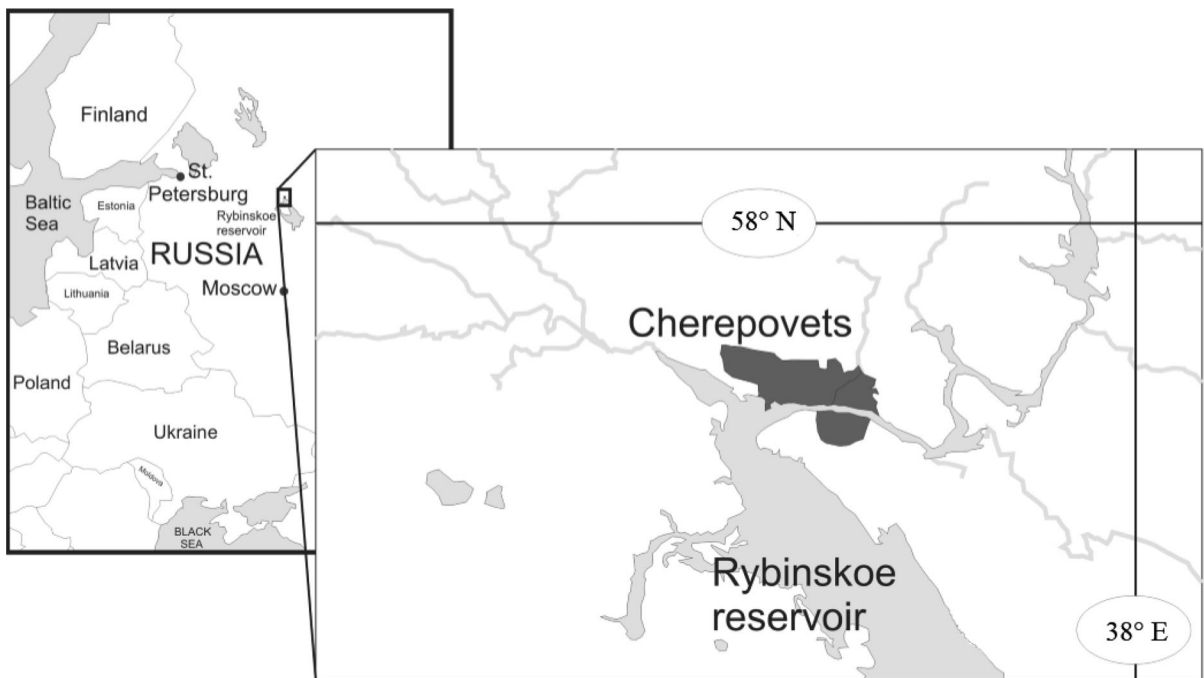


Fig. 1 The region of studies (Cherepovets, Vologda oblast, Russian Federation)

To analyze the mercury content, a strand of hair was taken from the root, from the back of the head and placed into paper envelopes. The envelope was attached to the survey participant's questionnaire. According to the United Nations Environment Program (UNEP), hair is the most convenient indicator of mercury intake in the body, including methylmercury in the process of its formation (UNEP 2002). The amount of mercury in the hair is proportional to its concentration in the blood. On average, the content of mercury in hair exceeds its amount in blood by 200–300 times (Committee on..., 2006). Hair analysis allows a quick and inexpensive qualitative assessment of the health of large populations, since the method is simple and noninvasive. WHO and IPEN recommend the use of hair to measure the mercury content in the organism (WHO 2003, Bell 2017).

Colored and gray hair is not excluded from the study sample.

The mercury content in the hair of women in the city of Cherepovets was determined in the ecological and analytical laboratory at the Department of Biology of the Cherepovets State University using the RA-915 M mercury analyzer with the PIRO-915+attachment by the atomic absorption pyrolysis method without preliminary sample preparation (detection limit 1.0–2.0 µg/kg). The accuracy of analytical methods of measurement was controlled using certified biological material DOLM-5 (Institute of Environmental Chemistry, Ottawa, Canada). The reproducibility of the method (coefficient of variation of repeated measurements) was from 0.05 to 2.5%.

Statistical analysis included a number of methods and was performed using the Statistica 12.0 and SPSS 17.0 programs. The normality of data distribution was verified using the Kolmogorov–Smirnov test. The studied samples were not always distributed normally; nonparametric methods were used to compare groups. Quantitative research data are presented as the arithmetic mean ± standard error, the median of the interquartile range (25th and 75th percentiles). Values of cardiometabolic risk factors and Hg in hair were compared using Mann–Whitney criteria; χ^2 criterion was used to compare categorical variables. The relationship between the concentration of Hg in the hair and cardiometabolic risk factors (systolic blood pressure, diastolic blood pressure, cholesterol, glucose) was evaluated using linear regression analysis. To determine the different levels of Hg in the hair, they

were classified by quartiles. To determine different levels of Hg in the hair, they were classified by quartiles (Q)—the variation series was divided into four equal parts. The median (Me) value of cardiometabolic factors was compared by the quartiles of mercury in the hair after adjusting for age, body mass index. The relationship between Hg levels and the presence of cardiometabolic risk was determined by analyzing the multiple logistic regression model on Hg quartiles and the results are presented as odds relationship (OR) and 95% confidence interval (CI). Regression analysis was adjusted for age and body mass index.

In all cases, the differences were considered statistically significant at $P \leq 0.05$.

Results

The average concentration of Hg in the hair of female residents of the city of Cherepovets is 434 ± 24 µg/kg (median=300, the interquartile range: 158–543). Minimum concentrations of mercury in hair were found in young women with a lower body mass index and rarely consuming fish and maximum in older women who consume fish often (see Supplementary Material Tables 1, 2 and 3). It was found that the average Hg content in the hair of women consuming fish two or more times a week is 615 ± 74 µg/kg being 4 times higher than in the hair of women who do not eat fish (168 ± 29 µg/kg) (Fig. 2). Fish from local waters may represent a more significant source of mercury in the body than marine fish (see Supplementary Material). The dependence of mercury content in the hair of women in the city of Cherepovets from such factors as their level of education, occupation, smoking, hair dyeing has not been established (see Supplementary Material).

The average age of women in the entire sample was 34 years. The age of women with metabolic syndrome (Mean – 39; min–max – 19–59) was significantly higher than that of the control group (Mean – 33; min–max – 18–58). The median values of mercury in hair, BMI, systolic blood pressure, DBP, cholesterol, glucose were statistically significantly higher in women with metabolic syndrome than in the control group (Table 1). In the group of women with metabolic syndrome, most common diagnosing criteria (cardiometabolic risk factors): BMI > 30 kg/m² registered

Table 1 Clinical characteristics of women with and without metabolic syndrome living in Cherepovets (n=387)

Indicators	Metabolic syndrome (+) (n=204)		Metabolic syndrome (-) (n=183)		P-value
	Median	25%–75%	Median		
Age (years)	35	30–45.6	32	0.001	0.001
Mercury content in hair (µg/kg)	360	190–580	260	<0.001	<0.001
systolic blood pressure (mmHg)	127.5	120–135	110	<0.001	<0.001
diastolic blood pressure (mmHg)	80	70–85	70	<0.001	<0.001
BMI (kg/m ²)	32.7	31.1–35.3	22.6	<0.001	<0.001
Cholesterol (mM/l)	5.4	5.2–5.8	4.4	<0.001	<0.001
Glucose (mM/l)	4.9	4.4–5.6	4.7	0.01	0.01

Blood pressure, mmHg: diastolic blood pressure ≥ 85; systolic blood pressure ≥ 130

Table 2 Cardiometabolic risk factors depending on quartiles (Q) of mercury content in hair

	Hg quartiles				P-value single logistic regressions	*P-value multiple logistic regression
	Q 1 ≤158 µg/kg Hg (n=97)	Q 2 159–299 µg/kg Hg (n=97)	Q 3 300–543 µg/kg Hg (n=97)	Q 4 ≥544 µg/kg Hg (n=96)		
systolic blood pressure (mmHg)	118.00 (109.00–125.50)	120.00 (110.00–128.00)	120.00 (110.00–131.00)	120.00 (110.00–130.00)	0.02	0.28
diastolic blood pressure (mmHg)	75.00 (70.00–80.00)	78.00 (70.00–80.00)	76.00 (70.00–81.50)	75.50 (70.00–84.00)	0.26	0.10
Cholesterol (mM/l)	4.96 (4.20–5.40)	5.00 (4.32–5.42)	5.30 (4.48–5.70)	5.38 (4.57–5.90)	<0.001	0.15
Glucose (mM/l)	4.67 (4.20–5.15)	4.80 (4.27–5.40)	4.90 (4.43–5.41)	5.11 (4.38–5.50)	0.006	0.78

n- number of women studied. *The analysis was adjusted for age and BMI

Table 3 Odds ratio of risk of metabolic syndrome among quartiles of mercury in hair

	Hg quartiles			
	Q 1 (n=97)	Q 2 (n=97)	Q 3 (n=97)	Q 4 (n=96)
Number (%)	43 (21.1%)	45 (22.1%)	57 (27.9%)	59 (28.9%)
OR	1	0.74	1.06	1.42
The analysis was adjusted for age and BMI	95% CI	(0.25–2.19)	(0.36–3.12)	(0.54–5.20)

with 99%, Cholesterol ≥ 5.2 mM/l – with 76%, systolic blood pressure ≥ 130 mmHg – with 46%. Diastolic blood pressure ≥ 85 mmHg registered with 34%, Glucose ≥ 5.6 mM/l – with 23% study participants. It

should be noted that for the entire sample and for each group separately, the average concentration of mercury in the hair (Mean – 430 µg/kg; with metabolic syndrome – 520, without metabolic syndrome – 330)

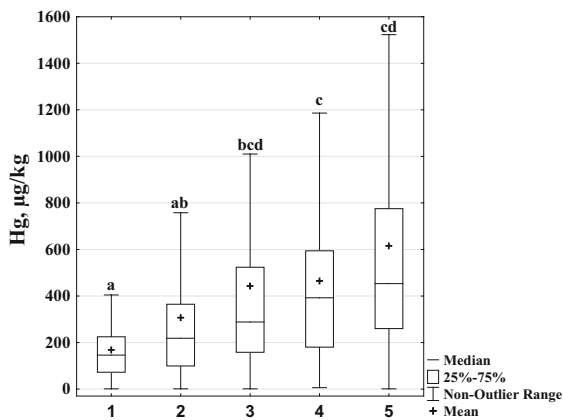


Fig. 2 The content of mercury in the hair of women with different frequency of fish consumption: 1—never, 2—less than 1 time a month, 3—1–2 times a month, 4—at least 1 time a week, 5—two or more times a week (a–d—different letter indices reflect statistically significantly different samples at a significance level of $p \leq 0.05$ (H-test—Kruskal–Wallis test)

was 1.5 times higher than median (Me – 290 $\mu\text{g}/\text{kg}$; metabolic syndrome – 360, without metabolic syndrome – 260).

The values of cardiometabolic risk factors (systolic blood pressure, diastolic blood pressure, cholesterol, glucose) did show a statistically significant increase when classifying Hg levels in hair by quartiles. At the same time after adjusting for age and body mass index, these differences are not statistically significant (Table 2).

Multiple logistic regression analysis of cardiometabolic risk factors in accordance with quartiles of Hg levels did not show a significant relationship between an increase in Hg levels and the development of cardiometabolic risk; however, the chances of meeting a woman with metabolic syndrome are higher in the highest mercury quartile (Table 3).

Discussion

Cherepovets city is a large industrial center of the North-West of Russia, where heavy and light industry enterprises are concentrated. Over 400 million tons of coal have been processed during the operation of the Plant with a full metallurgical production cycle, as a result of which approximately 950–1300 tons of mercury were released into the atmosphere (the concentration of mercury in coals—20–56 mg/kg

(Mukherjee 1999)). In addition, enterprises in other industries (chemical production of mineral fertilizers, mechanical engineering and metalworking enterprises, building materials enterprises, etc.) in Cherepovets may be potential sources of local release of mercury into the environment.

It is established that the average concentration of Hg in the hair of female residents of the city of Cherepovets is 434 ± 24 $\mu\text{g}/\text{kg}$ (median=300).

WHO recommends paying more attention to health status if the concentration of mercury in the hair exceeds 2 mg/kg (2000 $\mu\text{g}/\text{kg}$) (WHO 2003). However, studies conducted over the past 10 years in many countries have shown that health risks can occur at significantly lower metal concentrations. For women of reproductive age, the threshold level of the metal content should be 0.58 mg/kg (580 $\mu\text{g}/\text{kg}$) (Bell 2017).

The average concentration of mercury in the hair of residents of Cherepovets is 5 times lower than the threshold value set by the WHO (2000 $\mu\text{g}/\text{kg}$). The excess of this value was observed only in 2% of the examined women. At the same time, the mercury level exceeded 580 $\mu\text{g}/\text{kg}$ in 22% of the women examined.

Previous studies noted that the degree of industrial development of regions in the northwest of the Russian Federation is not a key factor affecting the level of mercury content in organs and tissues of living organisms of various taxonomic groups (Ivanova et al. 2014, 2020; Khabarova et al. 2018; Shuvalova et al. 2018).

Mercury concentrations in soils, earthworms and organs of small mammals from the vicinity of Cherepovets are 10–40 times lower than in the corresponding components of the ecosystems of industrial areas in Europe, the USA and China (Komov et al. 2016). The average mercury content in the organs of predatory mammals living in the vicinity of the city of Cherepovets does not exceed the US “standard for the health of the terrestrial ecosystem” – 1.1 mg/kg (Komov et al. 2016, 2012; Gerstenberger et al. 2006).

Mercury enters ecosystems mainly through atmospheric transport. The most significant role in the biogenic migration of mercury is assigned to the ecological and geographical (natural) conditions of the territory. Previously, it was shown that the level of accumulation of mercury in the body of people

depends on their socio-economic status and the amount of fish in the diet (WHO / UNEP 2008).

Residents of the Vologda Oblast consume fish at a rate of 23.3 kg/year per capita (ROSSTAT 2017) significantly less than the residents of the island states (Maldives—142.3 kg/year; Seychelles—58.3 kg/year; Japan—45.3 kg/year) and countries of Northern Europe and the European Mediterranean (30–57 kg/year). The amount of fish consumed by the residents of the Vologda Oblast is comparable to the average fish consumption by the population of North America (22.4 kg/year) and Australia (25.9 kg/year); 1.5–2 times higher than in Central Europe (5–17 kg/year), Latin America (10.5 kg/year) and Africa (9.9 kg/year) (FAO 2019).

Despite the presence of large enterprises of the chemical industry and metallurgy in the city of Cherepovets, the established concentrations of mercury in the hair of its residents are 2–3 times lower than the content of mercury in the hair of residents of neighboring settlements (situated 100–350 km away from industrial territories of Cherepovets) Babaevo town (mean=950 µg/kg), Vytegra town (mean=1080 µg/kg), Kirillov town (mean = 1200 µg/kg) (Maksimova 2016, Shuvalova and et al. 2018, Rumiantseva et al. 2018). For comparison with other regions of Russia, the average mercury content in the hair of women from the Eastern Siberia (Irkutsk region) – a region subject to industrial pollution, was 5310 µg/kg (Gorbachev 2016), 250 µg/kg in the Moscow Region (Egorov et al. 2014). Average mercury content in the hair of European women 240 µg/kg in the Czech Republic (Kruzikova et al., 2009), 190 µg/kg in United Kingdom, 110 µg/kg in Germany (Lindow et al. 2003; Angerer et al. 2017), 350 µg/kg in Sweden (WHO/UNEP 2008). At the same time, the average concentration of mercury in the hair of residents of the city of Cherepovets is an order of magnitude lower than the established concentration of mercury in the hair of women in the Pacific island states with a diet rich in fish (Solomon Islands, Marshall Islands, Kiribati, etc.), states where small-scale gold mining (artisanal gold mining) is carried out (Indonesia, Myanmar, Kenya) and states with industrial enterprises that are sources of mercury pollution (Thailand) (Bell 2017).

Studies in recent decades indicate that mercury is one of the risk factors for the development of cardiovascular diseases in the population (Choi et al.

2020; Genchi et al. 2017). It has been found that mercury inactivates a number of enzymes, antioxidants and amino acids with high affinity for sulfhydryl groups (Tchounwou et al. 2012), which negatively affects atherosclerotic cardiovascular diseases. The relationship between the content of mercury in the body and diseases of the coronary and peripheral arteries, hypertension, ischemic heart disease, myocardial infarction, occlusion of the carotid artery and atherosclerosis, as well as the effect of mercury on changes in blood pressure and heart rate variability (Asgary et al. 2017; Hu et al. 2018; Martins et al. 2018; Valera et al. 2012; Wells et al. 2017). The WHO notes that the level of mercury in the body is one of the reasons for the development of cardiovascular diseases (WHO 2010).

An analysis of the results in the study showed a statistically significant increase in mercury in the hair and BMI, SBP, DBP, glucose, cholesterol in women with metabolic syndrome compared with the control group. The values of SBP, DBP and total cholesterol have significantly increased in mercury quartiles in adolescent girls, after adjustment for age and anthropometric indices (Poursafa et al. 2014). Korean scientists showed a statistically significant increase in the body mass index, DBP, total cholesterol, depending on the level of mercury in the blood of adult Koreans after adjustment for covariates. In addition, exposure to Hg was significantly associated with the risk of metabolic syndrome (OR for metabolic syndrome in the highest quartile of mercury content was 1.68 (95% CI 1.25–2.25) compared with the lowest quartile) (Eom et al. 2014). In contrast, scientists from Finland have not found a correlation between mercury levels in hair and vascular reactivity, but note that exposure to mercury may increase the risk of hypertension (Bautista et al. 2009).

In this study, there was no statistically significant increase in cardiometabolic risk factors for quartiles of mercury in hair after adjusting for age and BMI. However, women in the highest quartile of mercury (> 544 µg/kg) had a significantly higher OR of the metabolic syndrome compared to the group with the lowest quartile of mercury content. The data obtained are consistent with the previously established threshold level of metal content in the hair of women of reproductive age (580 µg/kg) (Bell 2017). It should be noted that mercury is only one of the multiple factors that stimulate metabolic syndrome. However, it

appears at a statistically significant level even in the moderate zone of mercury accumulation in the hair. With the established values of Hg content in the hair of young and middle-aged women in Cherepovets, it was noted that the concentration of mercury in the hair of women with cardiometabolic syndrome significantly exceeds the concentration of mercury in the hair of women from the control group (without cardiometabolic syndrome).

Our results show that the risk of developing metabolic syndrome increases with increasing levels of mercury in hair. The mercury content in the body is an important, but not the only risk factor for the development of cardiovascular diseases. The individual risk of developing metabolic syndrome is calculated based on a set of indicators: cholesterol and glucose levels in the blood, arterial hypertension, body mass index.

Conclusion

In an area remote from the mercury geochemical zones, the content of mercury in hair exceeds the safety limit (580 $\mu\text{g}/\text{kg}$ is the threshold level of metal content in hair for women of reproductive age) in 20% of the women studied. The main factor determining the accumulation of mercury in the body is the consumption of fish. Freshwater fish from local waters may represent a more significant source of mercury in the body compared to marine fish. The content of mercury in the hair of women with metabolic syndrome is statistically significantly higher than that of women from the control group. When the content of mercury in the hair of women is above 544 $\mu\text{g}/\text{kg}$, the risk of developing cardiometabolic syndrome is statistically significantly higher. At the same time, an increase in the level of mercury in the body is primarily responded to by the indicator of diastolic pressure, to a lesser extent by indicators of the amount of cholesterol and glucose in the blood. Thus, one of the risk factors for the development of cardiometabolic syndrome in women is the increased content of mercury in their body.

Based on the fact that the content of metal in the hair of residents of some districts of the Vologda Oblast is significantly higher than that of residents of the city of Cherepovets, we can assume a more

alarming situation with the health of the population in these areas.

There were some limitations in this study. First, the number of subjects in each group was relatively small. In the present study, a subgroup analysis was performed with variables for age, education, occupation, diet, smoking, hair color and health indicators. There is the potential for unintended bias due to the relatively small number of subjects. In addition, the data were recorded only once for each person, which made it difficult to conclude that there was a temporary relationship between the level of mercury in the hair with the development of factors and the occurrence of cardiometabolic syndrome. To clarify the effect of mercury on the indicators of metabolic syndrome, data on the daily consumption of fish and n-3 polyunsaturated fatty acids (PUFAs) are needed, but this analysis was not carried out by us.

On the territory of Russia, studies of the content of mercury in the hair of the population are being carried out. At the same time, the assessment of the potential health effects of mercury exposure to one of the most vulnerable population groups (women of reproductive age), carried out in this study, is extremely relevant. Previously, no similar studies were conducted in Russia.

Author contributions Ivanova E.S. contributed to conception and design of the study, drafting of the manuscript. Shuvalova O.P. contributed to data analysis. Eltsova L.S. contributed to conducting research, statistical data processing. Komov V.T. contributed to critical review of the article for important intellectual content and final approval for the publication of the manuscript. Kornilova A.I. contributed to statistical data processing. Ivanova E.S., Shuvalova O.P., Eltsova L.S., Komov V.T., Kornilova A.I. carried out the key stages of the experiment.

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Data availability The authors confirm that the data supporting the findings of this study are available within the article its supplementary materials.

Declarations

Conflict of interest The authors declare no apparent or potential conflicts of interest related to the publication of this article.

Ethical approval The program of this study was discussed and approved by the Bioethics Commission of Cherepovets

State University and the Territorial Department of Health of the Vologda Oblast (No. 2–1 / 55, 18.01.2019).

Consent to Publish The participant has consented to the submission of the case report to the journal.

Consent to Participate Informed consent was obtained from all individual participants included in the study.

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